Expression of Pancreatic Endocrine Markers by Mesenchymal Stem Cells From Human Adipose Tissue

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ABSTRACT

Mesenchymal stem cells (MSCs) from human adipose tissue have a great potential for use in cell therapy due to their ease of isolation, expansion, and differentiation, besides the relative acceptance from the ethical point of view. Our intention was to isolate and promote in vitro expansion and differentiation of MSCs from human adipose tissue into cells with a pancreatic endocrine phenotype. Human adipose tissue obtained from patients undergoing abdominal dermolipectomy was digested with type I collagenase. MSCs isolated by plastic adherence and characterized by cytochemistry and FACS were expanded in vitro. MSC differentiation into an endocrine phenotype was induced over 2 to 4 months with high glucose (25 mmol/L) media containing nicotinamide, exendin-4, and 2-mercaptoethanol. Insulin and glucagon expressions were analyzed by immunofluorescence. Cells isolated from human adipose tissue and expanded in vitro expressed MSC markers as confirmed by FACS and cytochemistry. Insulin but not glucagon production by differentiated cells was demonstrated by immunofluorescence. MSCs isolated from human adipose tissue were induced to differentiate in vitro into an endocrine phenotype that expressed insulin.

THE SEARCH FOR A RENEWABLE SOURCE of cells with β-cell properties represents a promising therapeutic alternative for diabetes mellitus.[1] Mesenchymal stem cells (MSC) from human adipose tissue have great potential for use in cell therapy due to their ease of isolation, expansion, and differentiation. Compared with bone marrow adipose tissue can be easily harvested from adult subjects with a higher amount of MSC per gram of tissue.[2] Our intention was to isolate and promote in vitro expansion and differentiation of MSC from human adipose tissue into cells with a pancreatic endocrine phenotype.

METHODS

Human adipose tissues were obtained from patients undergoing abdominal dermolipectomy and providing prior written informed consent. The study protocol was approved by the university's ethics committee (protocol number 1437/2008).

MSC Isolation and Culture

Dissected tissue fragments yielded subcutaneous adipose tissue that was washed with phosphate buffered saline (PBS) and minced into small fragments before digested with a 1 mg/mL type I collagenase (Invitrogen Co, Grand Island, NY, USA) for 30 minutes at 37°C on a shaker. The digested tissue was passed through a 40-μm mesh filter, washed with PBS and centrifuged yielding a cell pellet that was resuspended in DMEM-F12 medium (Invitrogen Co) supplemented with 10% fetal bovine serum for plating onto culture flasks at a density of 1×10^5 cells/cm^2. Adherent cells expanded in the same medium were characterized for endocrine differentiation. Cell characterization was performed in a FACS Calibur equipment with anti-CD29, CD73, CD90, CD105, CD166, CD14, CD31, CD34, and CD45 antibodies (all obtained from Abcam PLC, Cambridge, UK). Cells were also induced to differentiate into adipocytic and osteocytic lineages using established protocols, followed by cytochemistry analysis.[3]

MSC Differentiation

Induction of MSCs into a pancreatic endocrine phenotype was performed essentially as described by Tang et al.[4] Briefly, MSCs

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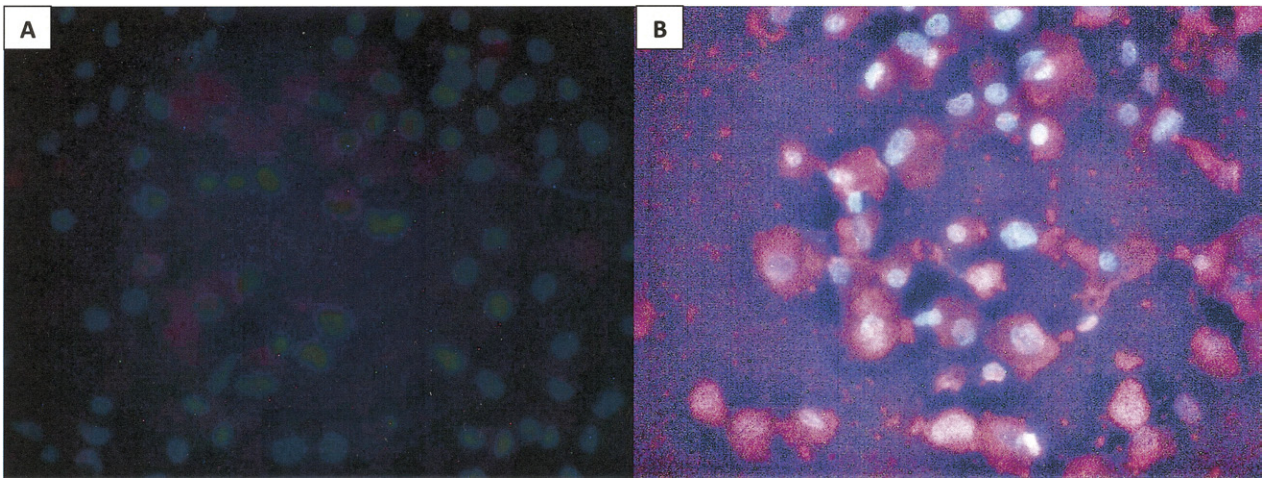

Fig 1. Immunofluorescence microscopy of human adipose tissue mesenchymal stem cells induced to differentiate during 4 months into a pancreatic endocrine phenotype. **(A)** Negative control. **(B)** Anti-insulin antibody + TR conjugated antibody. Nuclei stained with DAPI (×400).

were cultured for 2 to 4 months in H-DMEM (23 mmol/L glucose) + 10% fetal calf serum (FCS) until the appearance of cell clusters. The media were then changed to L-DMEM (5.5 mmol/L glucose) + 5% FCS + 10 mmol/L nicotinamide (Sigma-Aldrich, St Louis, Mo, USA) + 1 mmol/L 2-mercaptoethanol (Invitrogen Co) for 7 days. For the last 7 days they were cultured in the same media with added 10 mmol/L exendin-4 (Sigma-Aldrich).

Immunofluorescence

Cells from isolated clusters were disaggregated with trypsin, resuspended in PBS, and deposited onto positively charged glass slides by cytospin centrifugation. After fixation with 4% PFA, the cells were permeabilized with 1% Triton and incubated for 2 hours with primary antibodies against insulin and glucagon (Abcam PLC), followed by secondary Texas Red (for anti-insulin) or FITC (for anti-glucagon) conjugated antibody (Abcam PLC). Controls were performed with the absence of the primary antibody incubation. Nuclei were stained with DAPI. Slides were examined on a Nikon Eclipse E600 microscope.

RESULTS

Cells isolated from the human adipose tissue were mostly MSC as confirmed by FACS ($CD29^+$, $CD44^+$, $CD73^+$, $CD90^+$, $CD105^+$, $CD166^+$, $CD14^-$, $CD31^-$, $CD34^-$, and $CD45^-$) and cytochemistry (differentiated into adipogenic and osteogenic lineages). After 2 months of treatment with high glucose differentiation media, MSC in adherent cultures started to form cell clusters with islet-like appearances. Generally after 4 months treatment only clusters remained in the cultures. Immunofluorescence microscopy with cells obtained from these clusters showed production of insulin but not glucagon (Fig 1).

DISCUSSION

Due to their ease of isolation, expansion, and differentiation MSC from human adipose tissue are an interesting source for the generation of elements with β-cell properties. Adipose tissue can be obtained in large amounts from dermolipectomy and lipoaspiration procedures. MSC from human adipose tissue have shown the capacity to differentiate in vitro into insulin-producing cells.[5–7] In this work we sought to isolate MSC from human adipose tissue to promote its in vitro expansion and differentiation into cells with a pancreatic endocrine phenotype. We used a simple protocol that had already been employed with success to promote the differentiation of rat bone marrow MSC into insulin-producing cells. After 2 months' treatment the MSC cultures began to form cell clusters with an islet-like morphology; after 4 months' treatment, they produced insulin as shown by immunofluorescence microscopy. Insulin expression was confirmed by qPCR. However, differentiation into a β-cell phenotype was incomplete; glucagon was not produced, showing that our protocol needs some improvements.

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